THE PRESENCE OF SOIL AND FECAL STRAINS OF ORGANISMS OF THE COLON-AEROGENES GROUP IN THE WATERS OF KANSAS*

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It was demonstrated by Rogers, Clark, and Evans[1] that organisms in the colon-aerogenes group occurring on grains may be differentiated from those of fecal origin by the gas ratio. Clark and Lubs[2] showed that there is a complete correlation between the gas ratio and the hydrogen-ion concentration, the fecal strains in their media being characterized by a high hydrogen-ion concentration, the cultures from grains by a low hydrogen-ion concentration. The difference in H^+ concentration is easily recognized with methyl red as an indicator.

If organisms which apparently are of the colon-aerogenes group occur on grains and in soil, then their presence in water cannot be taken as evidence of fecal contamination unless it is proved that the fecal strains are more prevalent than the soil strains. It is the purpose of this paper to record the prevalence of the two strains of B. coli in ground waters and surface waters in the state of Kansas.

SOURCES OF CULTURES AND METHOD OF ISOLATION

Of the cultures studied, 247 were isolated from surface waters, 158 from ground waters, 19 from natural ice, and 8 from manufactured ice.

Both broth and bile fermentation tubes, made according to the Standard Methods, were used in the presumptive test for organisms of the colon-aerogenes group. From April 4 to May 4, 1916, a fermentation tube of every positive sample was saved and the sample streaked on Endo agar. Colonies having the appearance of B. coli were picked and streaked on agar slants. From these, transfers were made to dextrose, lactose, saccharose, and dulcite fermentation tubes, to the dipotassium-acid-phosphate medium of Clark and Lubs,[2] and to litmus milk and gelatin tubes. The fermentation tubes and milk tubes were incubated 2 days at 37 C., and the dipotassium-acid-phosphate medium was incubated 5 days at 30 C. One half of the latter was tested with methyl red and the other half with 10% KOH to obtain the Voges-Proskauer reaction. All cultures that did not ferment dextrose and lactose, produce acid, and coagulate milk, and which failed to liquefy gelatin, were discarded.

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[1] Jour. Infect. Dis., 1915, 17, p. 137.

[2] Ibid., p. 160.

CHARACTERISTICS OF THE CULTURES

The distribution of the cultures among MacConkey's[3] 4 principal groups and their relation to methyl red are shown in Table 1. This table shows 70% of the total number isolated to be acid to methyl red, that is, of fecal origin.

TABLE 1

DISTRIBUTION OF ORGANISMS ISOLATED FROM SURFACE WATERS AND GROUND WATERS

Organism	Number	Acid to Methyl Red	Percentage	Alkaline to Methyl Red	Percentage
B. communior	186	142	76	44	24
B. communis	50	44	89	6	11
B. aerogenes	112	54	49	58	51
B. acidi-lactici	57	42	74	15	26
Total	**405**	**282**	**70**	**123**	**30**

Table 2 shows the distribution of cultures isolated from raw water, including river, creeks, and impounding reservoirs.

TABLE 2

DISTRIBUTION OF ORGANISMS ISOLATED FROM RAW WATER

Organism	Number	Acid to Methyl Red	Percentage	Alkaline to Methyl Red	Percentage
B. communior	56	47	84	9	16
B. communis	15	13	87	2	13
B. aerogenes	29	18	62	11	38
B. acidi-lactici	16	10	63	6	37
Total	**116**	**88**	**76**	**28**	**24**

Table 3 shows the distribution of cultures isolated from treated waters. Most of these were waters filtered through mechanical filters; the others were waters settled with alum, or lime and alum only, or waters treated with hypochlorid.

TABLE 3

DISTRIBUTION OF ORGANISMS ISOLATED FROM TREATED WATER

Organism	Number	Acid to Methyl Red	Percentage	Alkaline to Methyl Red	Percentage
B. communior	62	50	81	12	19
B. communis	19	16	84	3	16
B. aerogenes	31	14	45	17	55
B. acidi-lactici	19	12	63	7	37
Total	**131**	**92**	**70**	**39**	**30**

[3] Elements of Water Bacteriology, 1915, p. 149.

A slightly higher percentage of the cultures from raw water were of fecal origin than of the cultures from treated waters. Samples of raw and treated waters were taken at the same time, but the raw waters were practically always positive in the presumptive test while many of the treated waters were negative. During the month that these cultures were isolated there was a high rainfall over almost the whole of Kansas. It would seem that there would be a higher percentage of B. coli of soil origin during high-water periods than during low-water periods.

Daily tests in the field are now being made at Cherryvale, Independence, Coffeyville, Chanute, Humboldt, and Washington, all surface supplies, to find whether one strain of B. coli is more resistant than another in the treated waters, and whether there is a recurrence of the organism in MacConkey's 4 principal groups.

Experiments, started more than 2 years ago, are being conducted in this laboratory to determine the longevity of organisms of the colon-aerogenes group in soil. Organisms of both soil and fecal origin are being used under both natural and artificial conditions.

TABLE 4

DISTRIBUTION OF ORGANISMS ISOLATED FROM ICE

Organism	Number	Acid to Methyl Red	Percentage	Alkaline to Methyl Red	Percentage
ORGANISMS FROM NATURAL ICE					
B. communior	10	3	100	0	70
B. communis	1	1	100	0	0
B. aerogenes	7	1	14	6	86
B. acidi-lactici	1	1	100	0	0
Total	19	6	32	13	68
ORGANISMS FROM MANUFACTURED ICE					
B. communior	2	1	50	1	50
B. communis	4	4	100	0	0
B. aerogenes	1	0	0	1	100
B. acidi-lactici	1	1	100	0	0
Total	8	6	75	2	25

Of the cultures from raw water 76% were of fecal origin, while of the organisms from natural ice only 32% were of fecal origin. Therefore if so small a number as 19 can be considered, it would seem that organisms of fecal origin are much shorter lived in ice than those of soil origin.

TABLE 5

DISTRIBUTION OF ORGANISMS ISOLATED FROM GROUND WATERS

Organism	Number	Acid to Methyl Red	Percentage	Alkaline to Methyl Red	Percentage
B. communior	68	45	66	23	34
B. communis	16	15	94	1	6
B. aerogenes	52	22	43	30	57
B. acidi-lactici	22	20	91	2	9
Total	**158**	**102**	**65**	**56**	**35**

Table 5 gives the distribution of 158 cultures isolated from ground waters. Sixty-five percent of the cultures were of fecal origin. Of the total, 123 were isolated from wells or springs in areas where the rock formation is limestone. Of these, 77% were of fecal origin. Thirty-four of the cultures were from wells or springs in areas where the rock formation is sandstone, shale, or glacial drift. Of these only 50% were of fecal origin.

CORRELATION OF THE METHYL RED AND VOGES-PROSKAUER REACTIONS

The work of Max Levine[4] on 9 cultures from sewage and 4 cultures from the American Museum of Natural History indicates that there is a complete correlation between the positive Voges-Proskauer reaction and the alkaline methyl-red reaction. The work I have done on cultures isolated from the waters and ices of Kansas agrees with his work. Of the cultures 138 were alkaline to methyl red, that is, they were of soil or grain origin, and in every instance they gave the positive Voges-Proskauer reaction. None of the cultures that were acid to methyl red, that is, of fecal origin, gave a positive Voges-Proskauer reaction.

CONCLUSIONS

The work on 247 cultures isolated from raw and treated waters indicates that a slightly higher percentage of the cultures from raw water were of fecal origin, than of the cultures from treated waters.

The work on 19 cultures from natural ices, as compared with 116 cultures from raw waters, would seem to indicate that colon bacilli of soil origin are more resistant to zero temperature than those of fecal origin.

[4] Jour. Infect. Dis., 1916, 18, p. 358.

A higher percentage of cultures of the colon-aerogenes group from wells and springs in limestone formation were of fecal origin than of those from wells and springs in sandstone, shale, and glacial drift. Too much emphasis should not be given this, however, since the number of wells in sandstone, shale, and glacial drift was limited.

The work on 405 cultures from surface and ground waters shows that 70% of the organisms of the colon-aerogenes group were of fecal origin.

There is complete correlation between the positive Voges-Proskauer reaction and alkalinity to methyl red.